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Scientific paper

Fertility of crossings, physiological potential and seed storage of sugar cane

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Abstract

Sugar cane (*Saccharum* sp.) is one of the main agricultural products in Brazil. For its cultivation it is used stems or rhizomes, which enable to reproduce the same genotype infinite times. However, in breeding programs, the sexual reproduction is necessary to produce new genetic combinations, and the use of seeds is the basis for obtaining more productive varieties. Thus, this study aimed to assess the fertility of bi-parental and multiple crossings, involving varieties RB92579 and RB92606, as well as to determine the physiological potential and storage of seeds (spikelets) produced. The determination of the fertility of crossings was performed by evaluating the percentage of caryopses produced in each crossing and the seed physiological potential was determined by the use of standard germination and vigor. To assess the potential of storage, seeds were kept during six months in kraft paper bags under cold room at 21 ± 4.5 ° C and relative humidity of 44± 14%, and the assessments conducted monthly by germination and vigor tests. The fertility and physiological potential of sugar cane seeds are strongly affected by the type of crossing involving varieties RB92579 and RB92606. Seed performance, despite minor fluctuations over time, was kept for six months of storage.

Keywords: genetic improvement, *Saccharum*, germination, vigor.

Introduction

Sugarcane (*Saccharum* sp.) has great importance to Brazil, which is the largest world producer of this raw material, and also is the largest exporter of the products sugar and alcohol. For a long time, sugarcane was considered one species of exclusively vegetative propagation, and it was believed that its spikelets did not produce seeds (CHASE and SENDULSKY, 1991). The obtaining of sugarcane plants trough seeds (spikelets) was a mark in this crop development, since it made possible the performance of the first works of genetic improvement, with the launch of new varieties, increasingly productive and adapted to unfavorable environmental conditions (BARBOSA et al., 2000; BARBOSA et al., 2002).

In the sugarcane genetic breeding programs, the production of seeds is performed using basically two types of breeding: bi-parental and multiple. In the bi-parental crossing, the hybridization is made between two genotypes of interest (CESNIK and MIOCQUE, 2004), while in the multiple crossing only the identity of the mother plant is known and the pollen come freely from different individuals (HEINZ and TEW, 1987). The effects of these crossing methods over the formation of viable seeds and/or with higher physiological potential are unknown, which enhances the need of investigation.

The production of seeds with high physiological potential represents the base of a good program of genetic breeding, since it is from them that it will be originated seedlings which may originate a new commercial variety (CHILTON et al., 1965), which may range between the different genetic materials and its hybrids since the physiological processes of seeds are genetically programmed and coded during its formation (MARCOS FILHO, 2005). To sugarcane, the production of seeds has not been satisfactory, since this crop is selected, among other purposes, to avoid the flowering, whose occurrence reduces its productive potential (CHASE and SENDULSKY, 1991; RODRIGUES, 1995).

Frequently in the programs of genetic

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breeding there is the necessity of storage of sugarcane seeds until the final period of cultivar selection. The physiological potential and the seed longevity are variables according to genetic characteristics and, the interaction of the air relative humidity and temperature, as well as the period of storage, is considered fundamental factors for the conservation of seeds (CARVALHO and NAKAGAWA, 2000).

RAJENDRA PRASAD and BALASUNDARAM (2006) verified conservation of the viability of sugarcane seeds when stored under temperature of -20 ºC, and the germination potential of seeds may be maintained up to five years. CUENYA et al. (1998) also observed higher conservation of seeds when under temperatures inferior to 0 ºC. However, there is little information about the behavior of seeds in function of the genotype, emphasizing the need of studies about appropriated conditions to storage.

Thus, considering the importance of sugarcane seeds for the genetic breeding program, the present work aimed at assessing the fertility of bi-parental and multiple crossings, involving the varieties RB92579 and RB92606 and at determining the physiological potential and storage of produced seeds.

Material and methods

The research was developed in the Laboratório de Análise de Sementes (Laboratory of Seed Analysis) and in the Galpão de Produção de Mudas (Shed of Seedling Production) which belong to the Programa de Melhoramento Genético da Cana-de-açúcar (PMGCA – Sugarcane Genetic Breeding), both located in the Centro de Ciências Agrárias of the Universidade Federal de Alagoas (CECA – Center of Agricultural Sciences/UFAL – Federal University of Alagoas), located in the municipality of Rio Largo, AL. Sugarcane seeds were obtained in the Estação de Floração e Cruzamento Serra do Ouro (EFCSO – Station of Flowering and Crossing Serra do Ouro), in Murici – AL.

For the evaluation of fertility and physiological potential of seeds, it was performed the bi-parental crossings RB92579 x RB92606 and RB92606 x RB92579, and the multiple RB92579 x ?, in two years of crossings (2006 and 2007). For the evaluation of the potential of storage of the seeds it was made the same crossings named earlier, adding the multiple crossing RB92606 x ?, made only in the crossing year 2006.

The fertility of each crossing was determined using four samples of 0.5 g of spikelets, and it was accounted the number of spikelets and the percentage of caryopses produced. The caryopses were extracted manually, with aid of a tweezers and a magnifier, taking off glumes, lemma and palea of the spikelets to obtain the raw caryopses.

For the determination of the physiological potential of seeds it was conducted experiments under controlled conditions (laboratory) and not controlled conditions (seedling production shed), using four replications of 200 spikelets for each crossing. In laboratory the spikelets were distributed between two paper towels moistened with distilled water in the proportion of 2.5 times the weight of the dry paper, conditioned in transparent plastic boxes (11 x 11 x 3.5 cm) and maintained in germination chamber (BOD type) regulated at temperature of 30 °C and photoperiod of 12 hours. In shed the spikelets were distributed in plastic trays, containing as substrate a mixture of ground, filter cake and coconut fiber, in the proportion 2:1:1. Next, spikelets were slightly wet to ease their adherence and they were maintained in room temperature. The evaluations were performed daily, during 21 days, calculating the percentage of normal seedlings, i.e., with all their essential parts visible (BRASIL, 2009). Having all the data, it was calculated the percentage and the germination velocity index (GVI), the latter, according to the formula proposed by MAGUIRE (1962).

For the evaluation of the potential of storage, the spikelets were kept for six months in kraft paper bags under refrigerated chamber at 21 ± 4.5 °C and air relative humidity of 44 ± 14%. It was performed monthly evaluations trough the germination test and germination velocity index, in laboratory and seedling production shed, described previously.

For the statistic analysis, it was used the completely randomized design. The data referent to the fertility of the crossings and physiological potential of the seeds were submitted to the analysis of variance, in factorial scheme 3 x 2 (3 crossings x 2 years of harvest). The means were compared by the Tukey test, at the level of 5% of probability. For the data of storage, it was used the Qui-square test, at the level of 1% probability.

Results and Discussion

The different crossings of sugarcane performed differed statistically from each other concerning the number of spikelets and caryopses produced. The crossing RB92579 x ? presented the highest percentage of caryopses formed for two years of harvest evaluated (Table 1) and, even though the crossing RB92579 x RB92606 obtained high average of spikelets in the crossing year 2006, this number did not imply in high production of caryopses, and it was registered only 3% of formed caryopses, with similar behavior in the year 2007.

Several factors may result in the reduction of the potential of seed production, which go from failures in the development of the anthers and of the ovules, incompatibility due to negative interactions between pollen and the pistil of the genotypes involved, as well as failures in the development of the embryo itself (BEWLEY et al., 2000). Besides that, the deficiency in the pollination is one of main factors involved in the reduction of the seed production, in grass until 40% of the florets may not be properly pollinated (FAIREY, 1993). According to MARTINS et al. (2009), the incidence of pathogens associated to determined crossings may limit the production of sugarcane seedlings, since they are benefited by the environmental conditions (high humidity and temperature) in which the seeds are produced, reducing thus the productive potential.

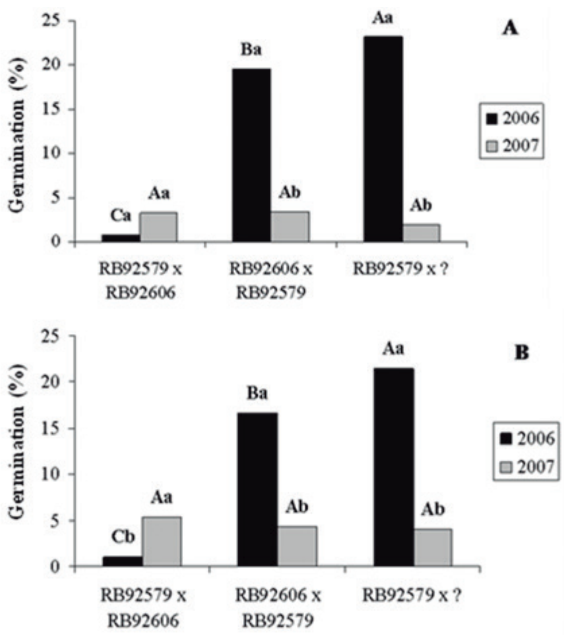
In relation to the spikelet germination, it was verified different responses between the crossings performed in 2006, either in controlled conditions or in non controlled conditions (Figure 1).

Besides that, the percentages of germination obtained from the crossings in 2006 were significantly

Table 1. Number of spikelets and percentage of caryopses of sugarcane, contained in samples of 0.5 g, coming from different crossings and years of harvest.

Crossing	Number of spikelets1		Caryopses (%)1	
	2006	2007	2006	2007
RB92579 x RB92606	1121.25 Aa	1173.25 Ba	3 Ca	7 Ba
RB92606 x RB92579	1034.75 Ba	1023.25 Ca	18 Ba	8 Bb
RB92579 x ?	1174.25 Ab	1413.50 Aa	25 Aa	19 Aa
CV (%)	3.84		24.31	

[1]Means followed by the same letter, uppercase in the column and lowercase in the line do not differ from each other by the Tukey test at the level of 5% of probability.



Equal uppercase letters between crossings and lowercase between crossing years do not differ by the Tukey test at the level of 5% of probability.

Figure 1. Germination (%) of seeds (spikelets) of sugarcane under controlled conditions (A) and non controlled conditions (B) coming from different crossings and harvest years.

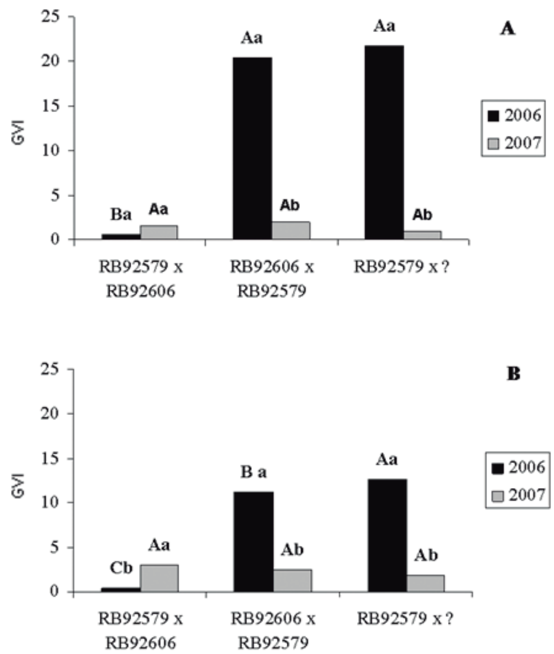
superior to those verified in the same crossings in 2007, with exception only of the spikelets obtained from the crossing RB92579 x RB92606. The difference in the germination response of sugarcane spikelets may be related to problems which occurred during the process of flowering, hybridization or seed maturation, which may affect the germination capacity of the produced seeds.

In the 2006 crossing, it was registered the highest percentage of spikelet germination obtained from the crossing RB92579 x ? in controlled and non controlled conditions (Figure 1), being coincident with the highest number of caryopses obtained by this crossing (Table 1) and presenting values close to the fertility. For the spikelets harvested in 2007 there was no effect of the type of crossing performed, which presented low production of seedlings in all crossings. This fact may have occurred due to the incidence of fungi in the crossing places (MARTINS et al., 2009), which may affect significantly the seed sanity and cause serious damages to the germination.

Conversely to the percentage of germination, in which it was verified significant differences between the crossings in controlled and non controlled conditions (crossing years 2006), the germination velocity index (GVI) was similar between the bi-parental crossing RB92606 x RB92579 and the multiple crossing RB92579 x ?, when in controlled conditions (Figure 2). In relation to the non controlled conditions, the differences were more contrasting between the three types of crossings, although with accented drop in the speed of seedling emergence, factor which may be related to the environmental variations, mainly temperature, which in some period of the day reached high values that may have caused damages to seeds.

Therefore, the spikelets obtained from the bi-parental crossing RB92606 x RB92579 and from the multiple crossing RB92579 x ?, harvested in 2006, presented highest vigor, independent on the type of environment. This fact may be related to the fact that the caryopses obtained from this crossings present a size visibly larger, since in several species the seed size is indication of the physiological potential (POPINIGIS, 1977), based on the principle that the largest the seed size, the highest amount of reserves are available for the nutrition of the embryo during the germination process (WULFF, 1995).

On the other hand, in the crossing year 2007, the seed vigor coming from the three crossings were



Equal uppercase letters between crossings and lowercase between crossing years do not differ by the Tukey test at the level of 5% of probability.

Figure 2. Germination velocity index (GVI) of seeds (spikelets) of sugarcane under controlled conditions (A) and non controlled (B), coming from different crossings and harvest years.

similar, verified by low germination velocity index.

Concerning the potential of seed storage, it was verified variation in the germination of different crossings and along the time, with small oscillations, and it is not possible to establish relation between germination and storage (Figure 3). This variability may have occurred due to the difficulty of homogenization of the samples.

In relation to the crossings, the spikelets obtained from the crossing RB92606 x ?, whose germination already presented superior value, tough it is worth to emphasize that the germination did not exceed 35%, showing that the sugarcane may present limitations concerning the production of viable seeds, which is notably inferior to the other grasses studied (FAIREY, 1993). According to CHASE and SENDULSKY (1991), this fact may be related to the process of selection which occurs in this species, directed to avoid the flowering.

The spikelets obtained from the crossing RB92579 x RB92606 presented values of germination considerably inferior in all the periods of storage, independent on the seeding conditions (controlled and non controlled) (Figure 3). This probably occurred due to the low potential of fertility presented by this crossing (Table 1).

According to MARTINS (2006), one limitation of the crossing between two varieties is the recombination, correspondent to the rearrangement of the genes during the meiosis for the formation of the gamete, since that few seeds are found with the desirable characteristics of the "parents", thus, great part of the seeds does not have the required quality and so it is performed thousands crossings to obtain seeds which may be selected, generation future commercial varieties.

Similar to the seed germination after the storage, the vigor evaluated by the GVI also differed between crossings, and there was variation over the storage periods (Figure 4).

When seeds were placed to germinate in favorable conditions of laboratory, it was possible to evaluate with more accuracy the vigor, reflecting the character inherent to the seed. On the other side, under non controlled conditions, in which there might occur fluctuations in the room conditions over time, the performance of seeds was inferior.

The germination and seed vigor, observed along the periods of storage studied, implies in the fact that in the storage of sugarcane seeds under temperature of 21 ± 4,5 °C and relative humidity of 44 ± 14% is capable to decelerate the process of

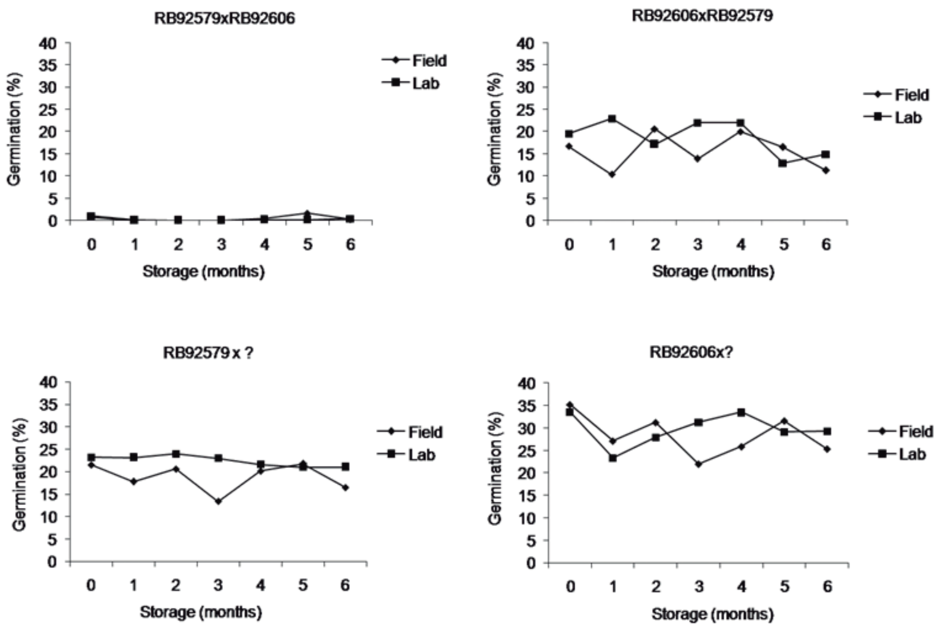


Figure 3. Germination (%) of seeds (spikelets) of sugarcane under controlled and non controlled conditions, coming from different crossings and submitted to the storage.

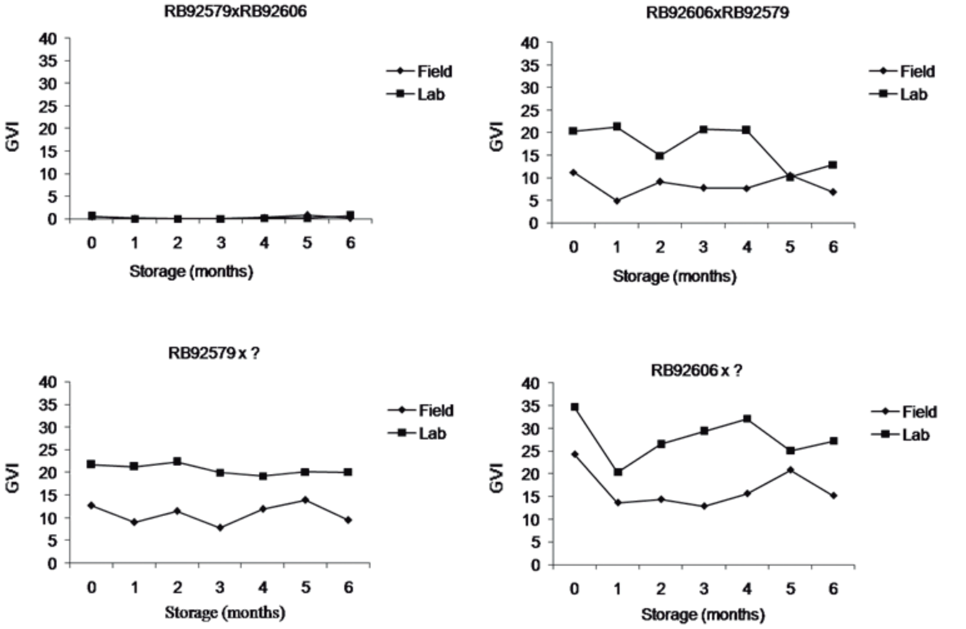


Figure 4. Germination velocity index (GVI) of seeds spikelets) of sugarcane under controlled and non controlled conditions, coming from different crossings and submitted to the storage.

seed deterioration, maintaining its physiological potential in until six months. Thus, the loss of the physiological potential may be minimized when in these conditions, when compared to the results found by RAO (1980), where there was loss of 90% of the germination in sugarcane seeds stored in room conditions of approximately 28 °C.

Conclusions

The potential of production of fertile spikelets and the physiological potential of sugarcane seeds (*Saccharum* sp.) may present variations in function of the type of crossing and in hybridizations performed in different years;

The multiple crossing RB92579 x ? enables the obtaining of higher percentages of fertile spikelets and of caryopses with highest physiological potential;

The performance of the seeds, despite presenting small oscillations along the time, may be conserved during six months of storage in refrigerated chamber at 21 ± 4,5 °C and air relative humidity of 44 ± 14%.

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